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Humoral Immunity in Arsenic-Exposed Children in Rural Bangladesh: Total Immunoglobulins and Vaccine-Specific Antibodies

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Background: Early-life arsenic exposure has been associated with reduced cell-mediated immunity, but little is known about its effects on humoral immunity.

Objective: We evaluated whether prenatal and childhood arsenic exposure was associated with humoral immune function in school-aged children.

Methods: Children born in a prospective mother–child cohort in rural Bangladesh were immunized with measles, mumps, and rubella (MMR) vaccines at 9 years of age ($n = 525$). Arsenic exposure was assessed in urine (U-As), from mothers during pregnancy and their children at 4.5 and 9 years of age. Total IgG (tIgG), tIgE, tIgA, and MMR-specific IgG concentrations were measured in plasma using immunoassays.

Results: Arsenic exposure was positively associated with child tIgG and tIgE, but not tIgA. The association with tIgG was mainly apparent in boys (p for interaction $= 0.055$), in whom each doubling of maternal U-As was related to an increase in tIgG by 28 mg/dL. The associations of U-As at 9 years with tIgG and tIgE were evident in underweight children (p for interaction <0.032). Childhood arsenic exposure tended to impair mumps-specific vaccine response, although the evaluation was complicated by high preimmunization titers. Postimmunization mumps–specific IgG titers tended to decrease with increasing U-As at 4.5 and 9 years of age [regression coefficient $(\beta) = -0.16$; 95% confidence interval (CI): -0.33, 0.01; $p = 0.064$ and $\beta = -0.12$; 95% CI: -0.27, -0.029; $p = 0.113$, respectively) in 25% children with the lowest preexisting mumps-specific IgG titers.

Conclusions: Arsenic exposure increased tIgG and tIgE in plasma, and tended to decrease mumps-specific IgG in children at 9 years of age. https://doi.org/10.1289/EHP318

Introduction

Exposure to inorganic arsenic through drinking water and certain food is a global public health concern. The arsenic problem is, perhaps, the most devastating in Bangladesh, where millions of hand-pumped tube wells yield drinking water with arsenic concentrations above the World Health Organization drinking water guideline value of 10 μg/L (WHO 2004). Chronic exposure to arsenic, a well-documented carcinogen (IARC 2012), has been associated with numerous noncancer effects, including immunotoxicity (Dangleben et al. 2013; Ferrario et al. 2016). In particular, arsenic seems to inhibit the proliferation of peripheral blood mononuclear cells as well as separated pan T cells, particularly T-regulatory cells, in response to specific mitogens as shown in both children and adults (Biswas et al. 2008; Hernández-Castro et al. 2009; Soto-Peña et al. 2006). We have also shown that prenatal arsenic exposure is inversely associated with placental T cells and thymic function in newborns (Ahmed et al. 2011; Ahmed et al. 2012; Raqib et al. 2009), and that childhood arsenic exposure is negatively associated with cell-mediated immune function (Ahmed et al. 2014), indicating arsenic-induced developmental immunotoxicity. Arsenic exposure may also impair the maturation, differentiation, and phagocytic function of macrophages as shown in arsenic-exposed adults with skin lesions compared to unexposed individuals (Banerjee et al. 2009). All these findings contribute to the growing evidence of increased risks of infectious diseases in relation to arsenic exposure, even at fairly low exposure levels (Farzan et al. 2016; Heaney et al. 2015; Rahman et al. 2010; Raqib et al. 2009; Smith et al. 2013).

Both T lymphocytes and macrophages are involved in the initiation of the humoral immune response by B lymphocytes (Abbas et al. 2012). Experimental studies on rodent splenocytes have shown that arsenic suppresses T-cell dependent antibody responses, as reviewed by Dangleben et al. (2013). Human data concerning the potential effects of arsenic on B cell-associated humoral immune function is, however, limited and inconclusive. Elevated concentrations of serum tIgG, tIgE, and tIgA were observed in arsenic-exposed Bangladeshi adults with skin lesions, compared to unexposed individuals (Islam et al. 2007), whereas no difference in cholera vaccine–specific IgG concentrations was found in children (2–5 years) living in high and low arsenic-exposed areas in Bangladesh (Saha et al. 2013). In this study we have followed up children born in a prospective mother–child cohort in Matlab, a rural area of Bangladesh with a wide range of arsenic exposure (Ahmed et al. 2014; Gardner et al. 2011). The aim was to evaluate whether prenatal and childhood arsenic exposure was associated with humoral immune function by measuring total plasma IgG, IgE, and IgA and measles, mumps, and rubella vaccine–specific plasma IgG concentrations following MMR vaccination in children at 9 years of age.

Materials and Methods

Study Area

The study was carried out in Matlab, a rural area of Bangladesh, where icddr,b has a health research and training center with a hospital and four subcenters. Here, icddr,b has been operating a health and demographic surveillance system (HDSS), covering a population of about 220,000 since 1966. Community health

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research workers update demographic and selected health information every 2 months.

The arsenic studies were initiated when it became apparent that elevated arsenic concentrations in groundwater were common in the study area (Rahman et al. 2006). More than 95% of the population use groundwater, retrieved from hand-pumped tube wells, as their main source of drinking water (icddr,b 2006). Screening of arsenic in all the functioning tube wells showed that 70% of the wells exceeded 10 μg/L and 30% exceeded 200 μg/L (Rahman et al. 2006; Vahter et al. 2006).

Study Design and Participants

The present study is part of our ongoing research in Matlab concerning the effects of arsenic and other contaminants in food and drinking water on pregnancy outcomes and child health and development (Ahmed et al. 2014; Gardner et al. 2013; Kippler et al. 2012; Raqib et al. 2009; Vahter et al. 2006). It was nested into a randomized population-based food and micronutrient supplementation trial among pregnant women: the Maternal and Infant Nutrition Interventions, Matlab (MINIMat trial, ISRCTN16581394), conducted between November 2001 and October 2003 (Persson et al. 2012). The enrolled women ($n = 4{,}436$) were randomly assigned to a daily dose of one of three different micronutrient supplementations: *a*) 30 mg iron and 400 μg folic acid (30Fe400F; iron dose matching the UNICEF preparation for multiple micronutrients (MM) supplementation), *b*) 60 mg iron and 400 μg folic acid (60Fe400F; the standard dose recommended by the Bangladesh government), or *c*) the UNICEF preparation of multiple micronutrients including 30 mg iron and 400 μg folic acid (MM group) (Persson et al. 2012). The MM group contained 15 different micronutrients (vitamins A, D, E, B1, B2, B6, B12, C, Niacin, Folic Acid and minerals Fe, Zn, Cu, I, Se) at the recommended daily allowance level, except folic acid, which was included at level of 400 μg. Women having facility-based deliveries between June 2003 and June 2004 were included in the current study (see Figure S1). From these women, we followed 640 mother–child pairs for studying immunotoxic effects of arsenic exposure in children at 4.5 years, and later at 9 years of age for the current study (see Figure S1). The selection of these 640 children was described in detail during the follow-up at 4.5 years of age (Ahmed et al. 2014). Since then, 42 children had migrated and 47 children were either absent ($n = 8$) or their parents did not give consent to participate ($n = 39$). Thus, 551 children participated in the present follow-up, and of these children, 26 either refused to donate blood or to take the MMR vaccine. The distribution of these children among the three arms of the supplementation was 181:181:163 (30Fe400F, 60Fe400F, and MM groups, respectively).

The study was approved by the Research Review and Ethical Review Committees at icbbr,b, Bangladesh, and the Regional Ethical Committee at Karolinska Institutet, Sweden. Written informed consent was obtained from the legal guardian of each child, and the child was free to refrain from any part of the study.

Assessment of Arsenic Exposure

We assessed individual arsenic exposure based on the concentrations of the sum of inorganic arsenic [arsenite (As^{III}), and arsenate (As^{V})] and its methylated metabolites [methylarsonic acid (MMA) and dimethylarsinic acid (DMA)] in urine, hereinafter referred to as urinary arsenic (U-As). Maternal urine in early pregnancy [on average in gestational week 8 (GW8)] and child urine at 4.5 years of age were collected as described previously (Ahmed et al. 2014; Vahter et al. 2006). Similarly, at 9 years of age, a spot urine sample was collected at the health clinics into a disposable metal-free plastic cup from which urine was transferred to a 24-mL polyethylene bottle tested essentially free from trace elements. All samples were kept in refrigerators until transferred, by the end of the day at the latest, to the Matlab hospital laboratory where they were stored at −70°C. The urine samples were transported frozen to Karolinska Institutet, Sweden, for the analysis of U-As.

The concentrations of arsenic metabolites in maternal and child urine were measured using high-performance liquid chromatography online with hydride generation and inductively coupled plasma mass spectrometry (HPLC-HG-ICPMS). All the measured arsenic concentrations in maternal and child urine were above the limit of detection, which was 0.2 μg/L for inorganic As^{III}, MMA and DMA, and 0.5 μg/L for inorganic As^{V}. The intra- and interassay coefficients of variation were similar, approximately 4%, based on the measurements of a reference urine sample (CRM No.18, National Institute for Environmental Studies, Tsukuba City, Japan), which was used for quality control purposes and measured together with the collected urine samples. The certified reference value of DMA was 36 ± 9 μg/L (mean ± SD) and our obtained DMA concentration was 43 ± 1.4 μg/L ($n = 96$), which is similar to that obtained previously (Ahmed et al. 2014; Gardner et al. 2011).

To compensate for variation in dilution, all the U-As concentrations were adjusted to the average specific gravity (1.012 at all-time points), measured by a digital refractometer (RD712 Clinical Refractometer; EUROMEX, Arnhem, the Netherlands) (Nermell et al. 2008).

Immunization by MMR

Under the national Expanded Program on Immunization (EPI), the studied children had been vaccinated after birth against tuberculosis, diphtheria, pertussis, tetanus, polio, measles, and hepatitis B (icddr,b 2014). For the purpose of the present study, we selected the MMR vaccine PRIORIX® (GlaxoSmithKline Biologicals SA, Rixensart, Belgium) because the children had not been vaccinated with rubella and mumps vaccines during infancy as these vaccines were not available in the EPI at that time. The MMR vaccine included live attenuated strains of Schwarz measles virus, the RIT 4,385 strain of mumps virus and the Wistar RA 27/3 strain of rubella virus. These three virus strains are mixed prior to lyophilization in a single dose. No other licensed vaccine, that would be naive and appropriate for this age group, was available. At the time of the follow-up, the children were vaccinated with MMR at the subcenter health clinics according to the manufacturer's recommendations.

Venous blood was collected in Na-heparin treated trace element–free tubes (Vacuette, Greiner Bio-One International AG, Kremsmünster, Austria) just before and 21 days after immunization. The blood samples were kept cold and transported within a couple of hours to the Matlab hospital laboratory, where plasma was immediately separated and stored at −70°C until transported frozen to the Dhaka laboratory for the analysis of total immunoglobulins (IgG, IgE, and IgA) and C-reactive protein in preimmunization plasma and MMR vaccine–specific plasma IgG in pre- and postimmunization plasma.

Plasma C-Reactive Protein (CRP)

We measured plasma concentrations of CRP as a marker of recent or ongoing infection in the children at 9 years of age. CRP was analyzed in the preimmunization blood plasma (neat) by Roche automated clinical chemistry analyzer (Hitachi 902, Mannheim, Germany), using a commercial kit [Tina-quant CRP (latex), Roche Diagnostic GmbH]. For quality control purposes, we used commercially available QC materials from Roche

Diagnostics. The limit of detection (LOD) for CRP was 0.03 mg/L, and all plasma samples were above this concentration. The interassay coefficient of variation was 6.2%.

Total Plasma IgG, IgA, and IgE Concentrations

Immunoturbidimetric assays (Tina-quant IgG Gen.2 kit and Tina-quant IgA Gen.2, Roche Diagnostic GmbH) were used for the quantitative determination of tIgG and tIgA in the preimmunization plasma in children at 9 years of age. Roche automated clinical chemistry analyzers, Hitachi 902 was used for tIgG with diluted plasma samples (1:16); and Cobas C311 was used for tIgA with neat plasma samples. For quality control purposes, we used commercially available QC materials. The interassay coefficient of variation for tIgG and tIgA was 2.2–2.4% and 2.8–4.5%, respectively. A sandwich immunoassay (Immuno CAP Total IgE, Phadia AB, Uppsala, Sweden) was used for the quantitative determination of total IgE in the preimmunization plasma. Phadia 250 automated laboratory system was used for this assay (Thermo Scientific, Phadia AB) with neat plasma samples. The intra- and interassay coefficients of variation were 3.5% and 3.8%, respectively.

In all plasma samples, total immunoglobulin concentrations were present well above the LOD (LOD for tIgG, tIgA, and tIgE was 44 mg/dL, 5 mg/dL and 0.1 IU/mL, respectively).

MMR Vaccine–Specific Plasma IgG Concentrations

Being live attenuated viral vaccines, the MMR vaccine generate T-cell dependent antibody response. Three separate enzyme immunoassays were performed for quantitative determination of measles, mumps, and rubella vaccine–specific IgG levels in pre- and postimmunization blood in children at 9 years of age, according to the manufacturer's instructions (IMMUNOLAB GmbH, Kassel, Germany). The plasma samples were diluted (1:101) before analysis. The LOD for measles, mumps, and rubella were 1.35 U/mL, 1.28 U/mL, and 0.29 IU/mL, respectively. The intra- and interassay coefficients of variation for all three vaccines were 4–9% and 5–11%, respectively.

Specific IgG concentrations against mumps and measles, >10 U/mL, and for rubella, >10 IU/mL, were defined as seropositive according to the manufacturer's recommendations (IMMUNOLAB). For evaluation of specific antibodies elicited after immunization, we defined a $\geq$2-fold increase in specific IgG response as seroconversion.

Covariates

Maternal anthropometry and parity, as well as socioeconomic status (SES) of the families were collected in early pregnancy at the enrollment in the MINIMat trial (Persson et al. 2012). SES was estimated via an asset index, generated through principal component analysis of household assets (Gwatkin et al. 2000). Anthropometry data were available for the children at 4.5 years of age (Ahmed et al. 2014). In the current study, children were examined by a general physician and were found to be healthy, without any history of immune-related diseases during enrollment. The families' SES was updated during the follow-up of the children at 4.5 and 9 years of age. Similar to 4.5 years of age, at 9 years of age, body weight was measured to the nearest 0.1 kg by a digital scale (TANITA HD-318; Tanita Corporation, Japan) with the children being barefoot and wearing light clothing. A standard weight of 20 kg was used to calibrate the digital scale regularly. Height was measured with a free-standing stadiometer [Leicester Height Measure (nearest to 0.1 cm; Seca 214, UK)]. The measured height and weight were converted to height-for-age, weight-for-age, and body mass index-for-age z-scores (SD scores), using the WHO growth reference for school-aged children and adolescents (De Onis et al. 2007). Season of birth and season of blood collection was categorized as pre-monsoon (January–May), monsoon (June–September), and post-monsoon (October–December).

Statistical Analysis

Statistical analyses were conducted using the software PASW 20.0 (SPSS Inc., Chicago, IL, USA) and Stata/IC, version 13.0 (StataCorp, College Station, Texas, USA). Data distribution patterns were evaluated using scatter plots, and normality and homogeneity of variances were formally checked by descriptive statistics. Associations between exposures, outcomes, and covariates were initially evaluated using Spearman's rank correlation coefficient (for continuous variables), Mann-Whitney U-test, analysis of variance, or Kruskal-Wallis test (for categorical variables), as appropriate.

To evaluate the association of prenatal (U-As at GW8) and childhood (U-As at 4.5 and 9 years of age) arsenic exposure with plasma total IgG, IgE, and IgA in the children at 9 years of age, we applied multivariable-adjusted linear regression. Exposure variables (U-As at GW8, 4.5 and 9 years of age) were $\log_2$-transformed to obtain normally distributed residuals with a homogeneous variance, and a simple interpretation of the beta-coefficient (average changes in outcome associated with each doubling of exposure). Regression models were adjusted for covariates that were significantly associated with both exposure and outcome or changed the effect estimate by 5% or more, that is, mother's BMI, mother's education, child age, HAZ at 4.5 or 9 years of age, sex, family SES at GW8 or 4.5 or 9 years of age, parity, season of blood collection, and plasma concentrations of CRP (listed in Tables 2–4) with the exact combination dependent on the time point of exposure used. Additionally, we included a multiplicative interaction term between urinary arsenic and sex or nutritional status (stunted and underweight) in the multivariable-adjusted regression models (p for interaction was considered significant if $p < 0.06$), and we repeated the above-mentioned analyses stratified by sex and nutritional status. In all cases, we used the best fit model on the basis of adjusted R^2 values. In sensitivity analysis, we additionally adjusted for birth weight and micronutrient supplementation groups.

The MMR-specific postimmunization plasma IgG in children at 9 years of age was evaluated in relation to arsenic exposures (U-As at GW8, 4.5 and 9 years of age) by quartiles of preimmunization MMR-specific plasma IgG levels. Since a large proportion of children had high preexisting antibody levels to measles, mumps, and rubella (>10 U/mL or 10 IU/mL) that may blunt the potential associations of arsenic with postimmunization antibody response to these vaccines, we focused on the individuals who were most naïve to the three viruses (first quartile) in the vaccine in order to have the greatest possibility to observe an exposure-response association. Both exposure and outcome variables were $\log_2$-transformed. The associations were adjusted for child HAZ, sex, SES, season of blood collection, and plasma concentrations of CRP.

Results

Demographic Data and Arsenic Exposure

The basic characteristics of the children at 9 years of age are shown in Table 1. The overall mean age was 8.9 years with a narrow range (8.6–9.6 years). The average height and weight were 124 cm and 22 kg, respectively, and girls were slightly shorter than boys ($p = 0.04$). About 19% of the boys and 23% of the girls were stunted, and 40% of the boys and 39% of the girls were underweight. The median U-As in the children (median 53 µg/L;

Table 1. Basic characteristics of the studied children at 9 years of age.

Variables[a]	All children ($n=525$)	Boys ($n=258$)	Girls ($n=267$)	p-Value
Age (years)	8.9 ± 0.12	8.9 ± 0.12	8.9 ± 0.11	0.861
Height (cm)	124 ± 5.4	124 ± 5.5	123 ± 5.2	0.040
HAZ	− 1.3 ± 0.9	− 1.3 ± 0.9	− 1.4 ± 0.87	0.164
Weight (kg)	22 ± 3.6	22 ± 3.4	22 ± 3.6	0.156
WAZ	− 1.7 ± 1.0	− 1.7 ± 1.1	− 1.7 ± 0.9	0.806
BAZ	− 1.3 ± 1.1	− 1.4 ± 1.1	− 1.3 ± 1.1	0.159
Family SES (at GW8)[b]	0.87 (− 5.8, 3.8)	0.93 (− 5.6, 3.8)	0.76 (− 5.8, 3.5)	0.636
Family SES (at 4.5 years)[b]	− 0.40 (− 3.5, 8.8)	− 0.44 (− 3.5, 8.8)	− 0.37 (− 3.5, 8.8)	0.820
Family SES (at 9 years)[b]	− 0.56 (− 6.1, 8.8)	− 0.55 (− 6.1, 8.8)	− 0.57 (− 6.1, 8.6)	0.893
Birth weight (g)	2,748 ± 391	2,795 ± 382	2,705 ± 396	0.010
U-As (μg/L)[c] at GW8	77 (2, 2063)	70 (2, 1,535)	92 (4, 2,063)	0.270
U-As (μg/L)[c] at 4.5 years	58 (12, 1,228)	57 (13, 1,123)	59 (12, 1,228)	0.104
U-As (μg/L)[c] at 9 years	53 (9, 1,268)	53 (9, 1,268)	54 (14, 647)	0.077
tIgG (mg/dL)	1,325 (144, 2,299)	1,300 (144, 2,299)	1,355 (636, 2,251)	0.028
tIgE (IU/mL)	733 (5, 11,929)	743 (16, 11,929)	729 (5, 9,367)	0.191
tIgA (mg/dL)	126 (31, 375)	129 (31, 375)	122 (44, 282)	0.365
Plasma CRP (mg/L)	0.37 (0.03, 22)	0.33 (0.03, 15)	0.41 (0.07, 23)	0.014

Note: BAZ, body mass index-for-age z-score; CRP, c-reactive protein; GW, gestational week; HAZ, height-for-age z-score; SES, socioeconomic status; U-As, Urinary arsenic; WAZ, weight-for-age z-score. p-Values were calculated using student t-test or Mann-Whitney U-test, as appropriate.
[a]Values shown are mean ± SD or median (range).
[b]SES score was estimated via an asset index, generated through principal component analysis of household assets.
[c]Adjusted to average specific gravity of 1.012.

range 9–1,268 μg/L; Figure 1) was significantly lower than that of their mothers during pregnancy (median 77 μg/L; range 2–2,063 μg/L; Figure 1; $p<0.001$), although the concentrations were correlated ($r_s=0.36$, $p<0.001$). There seemed to be no major change in the children's exposure since the previous follow-up at 4.5 years of age (median U-As 58 μg/L; range 12–1,228 μg/L; Figure 1; $r_s=0.56$, $p<0.001$). Over time the median exposure decreased to some extent but many children still had elevated exposure (Figure 1). Exposures (U-As at GW8, 4.5 and 9 years of age) and outcomes (tIgG, tIgE, tIgA) did not vary in relation to the supplementation groups (see Table S1).

Relation of Plasma CRP, Immunoglobulins and Arsenic Exposure

The median plasma CRP concentration was higher in girls than in boys (Table 1). Plasma CRP was weakly positively correlated with plasma tIgG ($r_s=0.21$, $p<0.01$), but not with tIgE ($r_s=0.05$, $p=0.24$) or tIgA ($r_s=0.01$, $p=0.84$). We did not observe any significant correlation between arsenic exposure and plasma CRP at any time. Plasma tIgG was weakly correlated with tIgE ($r_s=0.25$, $p<0.01$) and tIgA ($r_s=0.18$, $p<0.01$).

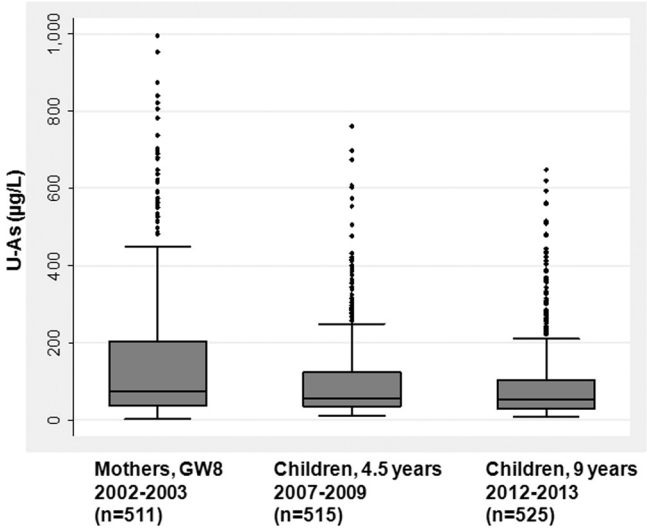

Figure 1. Box plot representing urinary arsenic concentrations in maternal urine at GW8, and children's urine at 4.5 and 9 years of age, limiting the x-axis at 1,000 μg/L of urinary arsenic.

Relation of Arsenic Exposure with Plasma Immunoglobulins

The average (mean ± SD) plasma concentration of tIgG, tIgE, and tIgA was 1,339 ± 260 mg/dL, 1,295 ± 1,507 IU/mL and 132 ± 45 mg/dL, respectively, in the children at 9 years of age. Boys had slightly lower tIgG than girls ($p=0.028$), but otherwise sex differences were small (Table 1).

In the multivariable-adjusted linear regression analysis, maternal U-As at GW8 was significantly positively associated with tIgG and tIgE (Table 2, Figure 2A,B). Sex was influential in the models and the interaction between U-As at GW8 and sex was significant in the full model with tIgG (p for interaction 0.055). When stratifying the analyses by sex, a significant positive association with tIgG was obvious only in boys (Table 2). Each doubling of maternal U-As was associated with 28 mg/dL (corresponding to ∼0.11 SD) increase of tIgG in the boys. The estimates for tIgG were similar with all three time points of exposure (U-As at GW8, 4.5 and 9 years of age) in all children (Table 2, Figure 2C,E). However, in the boys, the estimate for tIgG was slightly higher (∼20%) with prenatal exposure than with childhood exposures (Table 2). The beta estimate for tIgE increased with respect to arsenic exposure time points (β = 79, 94 and 110 for U-As at GW8, 4.5 and 9 years of age) (Table 2, Figure 2D,F). For instance, the estimate for tIgE for U-As at 9 years was 39% higher compared to the estimate for prenatal exposure (age of exposure), and again we observed relatively stronger associations in the boys than girls (Table 2), although the interaction term was not significant in the full models (p for interaction 0.181–0.314). We did not observe any significant association between U-As at any time point and tIgA (Table 2). As the nutritional status may affect the immune response, we also stratified the association between U-As (U-As at GW8, 4.5 and 9 years of age) and plasma immunoglobulins (tIgG, tIgE, and tIgA at 9 years of age) by stunting and underweight (Table 3). This showed stronger associations of U-As at 9 years with tIgG and tIgE in underweight children (p for interaction was 0.032 and 0.030, respectively) compared to children with adequate weight

Table 2. Multi-variable adjusted linear regression analysis of arsenic exposures (log_2-transformed U-As) with plasma tIgG, tIgE, and tIgA in children at 9 years of age.

Exposure	All children β (95% CI)[f]; *p*-value	*p* for interaction	Boys β (95% CI)[f]; *p*-value	Girls β (95% CI)[f]; *p*-value
U-As (μg/L) at GW8 ($n=511$)[a]				
tIgG (mg/dL)	17 (3.1, 30); 0.016	0.055	28 (7.1, 50); 0.009	5.1 (−13, 23); 0.570
tIgE (IU/mL)	79 (2.3, 156); 0.044	0.707	50 (−73, 173); 0.426	82 (−13, 178); 0.090
tIgA (mg/dL)	1.2 (−1.2, 3.7); 0.318	0.160	3.1 (−0.76, 6.9); 0.117	−0.70 (−3.8, 2.4); 0.658
U-As (μg/L) at 4.5 years of age ($n=515$)[b]				
tIgG (mg/dL)	15 (−1.9, 33); 0.081	0.384	23 (−4.2, 49); 0.098	11 (−12, 34); 0.364
tIgE (IU/mL)	94 (−5.6, 193); 0.064	0.253	148 (−6.8, 303); 0.061	40 (−86, 167); 0.531
tIgA (mg/dL)	−2.4 (−5.4, 0.73); 0.134	0.354	−1.2 (−6.1, 3.6); 0.613	−4.0 (−8.1, 0.04); 0.052
U-As (μg/L) at 9 years of age ($n=525$)[c]				
tIgG (mg/dL)	15 (−1.7, 32); 0.078	0.464	23 (−2.5, 49); 0.077	8.2 (−15, 31); 0.479
tIgE (IU/mL)	110 (13, 206); 0.026	0.196	171 (26, 317); 0.021	38 (−86, 162); 0.544
tIgA (mg/dL)	−1.1 (−4.1, 1.9); 0.469	0.299	0.24 (−4.3, 4.8); 0.915	−2.5 (−6.5, 1.5); 0.224
Combined exposure ($n=500$)[d,e]				
U-As (μg/L) at GW8				
tIgG (mg/dL)	14 (−0.93, 29); 0.066	0.115	24 (0.20, 47); 0.048	3.4 (−16, 23); 0.737
tIgE (IU/mL)	43 (−42, 129); 0.322	0.181	−28 (−165, 107); 0.678	86 (−21, 194); 0.114
U-As (μg/L) at 4.5 years				
tIgG (mg/dL)	0.59 (−21, 22); 0.958	0.962	2.7 (−30, 36); 0.872	5.3 (−25, 35); 0.735
tIgE (IU/mL)	50 (−76, 175); 0.438	0.408	99 (−94, 291); 0.316	18 (−148, 185); 0.828
U-As (μg/L) at 9 years				
tIgG (mg/dL)	11 (−10, 32.7); 0.313	0.556	20 (−11, 51); 0.218	1.4 (−29, 32); 0.929
tIgE (IU/mL)	60 (−63, 183); 0.337	0.314	136 (−45, 318); 0.141	−15 (−182, 151); 0.854

Note: U-As, sum of urinary arsenic metabolites; β, regression coefficient; CI, confidence interval; GW, gestational week; SES, socioeconomic status; tIg, total plasma immunoglobulin.
[a]Adjusted for child age, HAZ, sex (except when stratified by sex), SES (at GW8), parity, season of blood collection, mother's education, plasma CRP, and mother's BMI at GW8.
[b]Adjusted for child age, sex (except when stratified by sex), SES (at 4.5 years), parity, season of blood collection, mother's education, plasma CRP, and HAZ at 4.5 years of age.
[c]Adjusted for child age, HAZ, sex (except when stratified by sex), SES (at 9 years), parity, season of blood collection, mother's education, and CRP.
[d]U-As concentrations at GW8, 4.5 and 9 years of age were entered in the same regression model.
[e]Adjusted for child age, HAZ, sex (except when stratified by sex), SES (at 9 years), parity, season of blood collection, mother's education, CRP and mother's BMI at GW8.
[f]Average changes in outcome with each doubling of exposure.

(Table 3). The association of U-As at 4.5 years with tIgG was also evident in underweight children (*p* for interaction 0.026). The associations of tIgE with U-As at GW8 and 4.5 years were stronger in stunted children (*p* for interaction was 0.025 and 0.023, respectively) compared to children with adequate height (Table 3). We did not observe any significant influence of the relative fraction of the different arsenic metabolites in urine (% inorganic As, MMA or DMA) on the associations of arsenic exposures with tIgG, tIgE, or tIgA (data not shown). Also we did not observe any significant influence of supplementation groups on the association between arsenic exposure and tIgG, tIgE, or tIgA (data not shown).

To evaluate which temporal window of arsenic exposure (U-As at GW8, 4.5 and 9 years of age) influenced tIgG and tIgE the most, we entered all exposures in the same linear regression model (Table 2). Because the exposures were intercorrelated, we checked the variance inflation factor and it was <1.90 for all exposures. For the total IgG, the results indicated that the estimate for prenatal exposure decreased by 15% and were marginally significant ($p=0.066$) in the model, whereas the estimate for concurrent exposure (9 years) decreased by 27% and was not significant in the model. The estimate for U-As at 4.5 years decreased markedly and was not significant in the model. For total IgE, the estimates for all exposures decreased and were not significant in the models (Table 2).

Relation of Arsenic Exposure with MMR Vaccine–Specific Plasma IgG

The descriptive statistics of MMR vaccine–specific plasma IgG are presented in Table S2. All children had high preexisting antibody titers against all the three vaccines, indicating prior exposure to these viruses. For mumps and measles, 100% children were seropositive (>10 U/mL); whereas for rubella, 76% were seropositive (>10 IU/mL). The overall seroconversion after immunization (≥2-fold increase) for mumps, measles, and rubella were 78%, 57%, and 49%, respectively.

The fold changes of vaccine-specific IgG titers (postimmunization IgG/preimmunization IgG) for all three vaccines (measles, mumps, and rubella) were strongly negatively associated with the preimmunization vaccine–specific IgG titers (see Figures S2–S4). Therefore, we evaluated postimmunization measles or mumps or rubella-specific IgG titers in relation to arsenic exposure in quartiles of preimmunization vaccine–specific IgG titers. In the first quartile with lowest preimmunization mumps–specific IgG titers, postimmunization mumps–specific IgG titers decreased with increasing U-As both at 4.5 and 9 years of age (Table 4). The associations became stronger after adjusting for child HAZ, SES, season of blood collection, sex, and plasma CRP (Table 4, Model 1). When we additionally adjusted for the preimmunization mumps–specific IgG titers, the estimates attenuated by 15–25% (Table 4, Model 2). Maternal U-As at GW8 also showed an inverse association with postimmunization mumps–specific IgG, but the association was not statistically significant (Table 4).

We did not observe any significant association between U-As at any time point and postimmunization mumps–specific IgG titers in the higher quartiles of preimmunization IgG titers (data not shown). Also, we did not observe any significant association between U-As at any time point and the postimmunization measles or rubella-specific plasma IgG titers, not even in the children with the lowest preimmunization titers (see Table S3).

Discussion

The findings of the present study suggested that the concentrations of plasma tIgG and tIgE, but not tIgA, were higher with higher arsenic exposure in school-aged children in rural Bangladesh. Interestingly, the associations with tIgG was mainly apparent in boys, and the associations with both tIgG and IgE were apparent in underweight children. We also found an

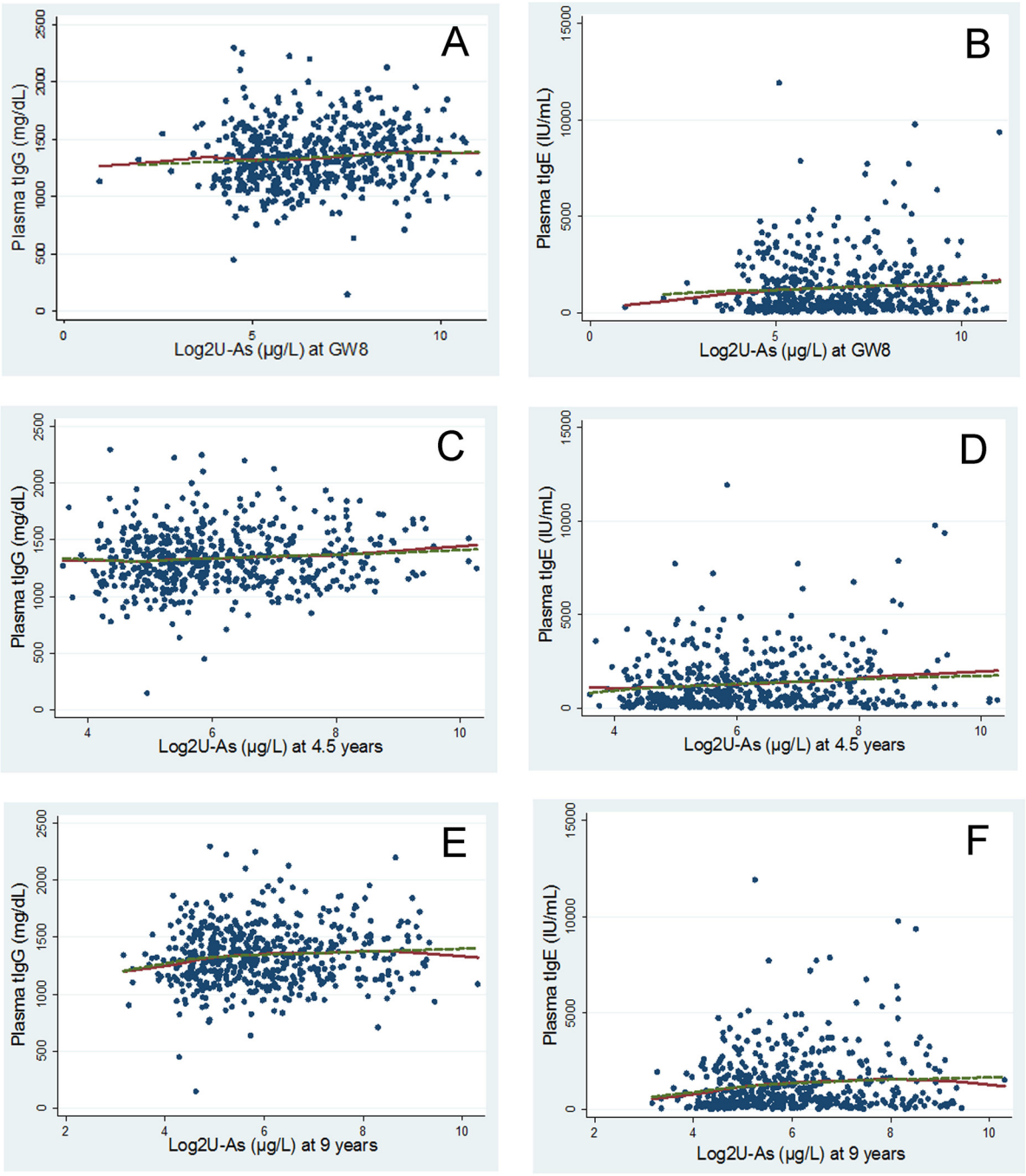

Figure 2. Associations of U-As at GW 8 with plasma tIgG (Figure 2A) and tIgE (Figure 2B). Associations of U-As at 4.5 years of age with plasma tIgG (Figure 2C) and tIgE (Figure 2D). Associations of U-As at 9 years of age with plasma tIgG (Figure 2E) and tIgE (Figure 2F). In the scatter plots, the solid line represents a Lowess (locally weighted scatter plot smoothing) moving-average curve for the raw data and the dashed line represents the fitted curve, adjusted for child age, HAZ, sex, SES, parity, season of blood collection, mother's education, plasma CRP, and maternal BMI at GW8.

indication of decreasing IgG response to mumps vaccine with increasing childhood arsenic exposure.

Plasma B cells synthesize and secrete IgG, IgA, and IgE, which constitute 80%, 15%, and 0.05%, respectively, of the total serum immunoglobulins in humans (Abbas et al. 2012). Generally, IgG is involved in controlling pathogenic organisms in the circulation and tissue fluids by different mechanisms, including neutralization of pathogens and their toxins, and induction of complement fixation (Abbas et al. 2012). However, elevated concentrations of plasma IgG have been associated with chronic or recurrent bacterial/viral infections as well as autoimmune/inflammatory diseases in children (Lo et al. 2013). A recent study in Bangladesh showed a positive association between total urinary arsenic in late pregnancy and maternal serum IgG (Ser et al. 2014). Our present finding of elevated tIgG in arsenic-exposed children could potentially contribute to conditions of arsenic-induced persistent immune activation/inflammation or autoimmunity, although this remains to be shown. The prenatal exposure showed the strongest association with tIgG, especially in the boys. The IgE is involved with the immunity against parasitic infection and associated with type 1 hypersensitivity, including allergic asthma, rhinitis, and food allergies

Table 3. Multi-variable adjusted linear regression analysis of arsenic exposure (log_2-transformed U-As) with plasma tIgG, tIgE, and tIgA stratified by stunting and underweight.

Exposure	Stunted β (95% CI)[d]; *p*-value	Adequate height β (95% CI)[d]; *p*-value	*p* for interaction	Underweight β (95% CI)[d]; *p*-value	Adequate weight β (95% CI)[d]; *p*-value	*p* for interaction
U-As (μg/L) at GW8 ($n=511$)[a]						
tIgG (mg/dL)	21 (−7.5, 49); 0.148	17 (1.8, 32); 0.028	0.859	26 (3.4, 48); 0.024	9.9 (−7.2, 27); 0.256	0.243
tIgE (IU/mL)	197 (0.56, 394); 0.049	43 (−40, 126); 0.314	0.025	97 (−25, 219); 0.120	65 (−38, 168); 0.216	0.713
tIgA (mg/dL)	4.5 (−1.34, 10); 0.129	0.57 (−2.1, 3.2); 0.675	0.155	1.01 (−3.2, 5.2); 0.640	0.83 (−2.1, 3.7); 0.576	0.819
U-As (μg/L) at 4.5 years ($n=515$)[b]						
tIgG (mg/dL)	21 (−17, 59); 0.282	12 (−7.15, 31); 0.217	0.602	37 (8.18, 64); 0.012	−2.6 (−24, 19); 0.813	0.026
tIgE (IU/mL)	302 (44, 560); 0.022	39 (−67, 144); 0.472	0.023	164 (7.41, 320); 0.040	34 (−96, 164); 0.609	0.211
tIgA (mg/dL)	1.47 (−6.3, 9.2); 0.71	−3.2 (−6.6, 0.09); 0.057	0.084	−0.19 (−5.6, 5.2); 0.944	−4.2 (−7.8, −0.60); 0.022	0.216
U-As (μg/L) at 9 years ($n=525$)[c]						
tIgG (mg/dL)	3.8 (−32, 40); 0.822	17 (−2.1, 36); 0.082	0.575	40 (9, 70); 0.010	−0.036 (−20, 20); 0.997	0.032
tIgE (IU/mL)	183 (−65, 431); 0.147	80 (−23, 184); 0.128	0.534	235 (71, 398); 0.005	37 (−85, 160); 0.552	0.030
tIgA (mg/dL)	4.7 (−2.5, 12); 0.197	−2.6 (−6.02, 0.73); 0.124	0.027	1.1 (−4.7, 6.9); 0.706	−2.2 (−5.7, 1.2); 0.209	0.313

Note: U-As, sum of urinary arsenic metabolites; β, regression coefficient; CI, confidence interval; GW, gestational week; tIg, total plasma immunoglobulin.
[a]Adjusted for child age, sex, SES (at GW8), parity, season of blood collection, mother's education, plasma CRP, and maternal BMI at GW8.
[b]Adjusted for child age, sex, SES (at 4.5 years), parity, season of blood collection, mother's education, and plasma CRP.
[c]Adjusted for child age, sex, SES (at 9 years), parity, season of blood collection, mother's education, and plasma CRP.
[d]Average changes in outcome with each doubling of exposure.

(Gould et al. 2003). The National Health and Nutrition Examination Survey showed a positive association between U-As metabolites and food sensitization, particularly IgE against egg, peanuts, and shrimp (Shiue 2013). In line with this, we also found a positive association between arsenic exposure, particularly in childhood, and tIgE. This might be an underlying contributor to the development of allergic diseases; however, we did not measure allergen-specific plasma IgE. The observed increase in the estimate with increasing time and degree of arsenic exposure on tIgE, indicates that long-term arsenic exposure may increase the susceptibility to allergic diseases or parasitic infections. We did not find any association between arsenic exposure and tIgA; it is possible that there is a lack of influence of arsenic on mucosal immunity. However, further research is necessary in this area to confirm the postulation.

Table 4. Regression analysis of associations between arsenic exposures (log_2-transformed U-As) and mumps-specific postimmunization plasma IgG (log_2-transformed) in the lowest quartile of mumps-specific preimmunization plasma IgG concentrations.

Exposure	Lowest quartile of preimmunization mumps–specific IgG (U/mL); median (range), 508 (243–1455) β (95% CI); *p*-value
U-As (μg/L) at GW8 ($n=128$), crude model	−0.042 (−0.18, 0.09); 0.548
Adjusted model 1	−0.015 (−0.16, 0.13); 0.831
Adjusted model 2	0.013 (−0.12, 0.14); 0.841
U-As (μg/L) at 4.5 years of age ($n=129$), crude model	−0.17 (−0.34, 0.012); 0.067
Adjusted model 1	−0.20 (−0.38, −0.008); 0.041
Adjusted model 2	−0.16 (−0.33, 0.01); 0.064
U-As (μg/L) at 9 years of age ($n=131$), crude model	−0.14 (−0.29, 0.02); 0.077
Adjusted model 1	−0.17 (−0.33, −0.003); 0.046
Adjusted model 2	−0.12 (−0.27, 0.029); 0.113

Note: U-As, sum of urinary arsenic metabolites; β, regression coefficient; CI, confidence interval; GW, gestational week. Model 1 Adjusted for child HAZ (exposure time specific), SES (exposure time specific), season of blood collection, sex, and plasma CRP. Model 2 Additionally adjusted for preimmunization plasma IgG concentrations.

A possible mode of action for the observed increase in child tIgG and tIgE in relation to arsenic exposure could be a compensation for an arsenic-induced reduction of T cells. In our previous studies, we showed that arsenic exposure during pregnancy suppressed T cells in the placenta and cord blood (Ahmed et al. 2011; Ahmed et al. 2012). We also observed that childhood arsenic exposure reduced T-cell–associated function in the same children when they were at 4.5 years of age (Ahmed et al. 2014). Therefore, in a state of T-cell paucity or impaired T-cell function, it is possible that the immune system compensates the T-cell void by polyclonal production of excessive levels of nonspecific antibodies (Montes et al. 2007). The scenario can be compared with HIV-infected children who have increased production of IgG, IgA, and IgE that do not confer protection against HIV, in presence of $CD4^+$ T-cell depletion (Shearer et al. 2000). During healthy pregnancy, the immune system changes towards higher Th2 cytokine levels (Calleja-Agius and Brincat 2008) and, as a result, the child is born with Th2 dominated immune response. After birth, the immune system requires maturation of the Th1 cytokine response to achieve effective host resistance to infections (Dietert and Zelikoff 2008). Arsenic exposure during pregnancy might disrupt this series of pre-and postnatal events. Thus, another reason could be an arsenic-induced imbalance between Th1 and Th2 cytokines (Ahmed et al. 2014; Morzadec et al. 2012; Soto-Peña et al. 2006), which might result in upregulation of the nonspecific antibody production, although, we did not measure IgG2a and IgG1 isotypes.

Our finding contributes to the growing body of evidence that some effects of arsenic are sex dependent (Broberg et al. 2014; Dangleben et al. 2013; Ferrario et al. 2016; Kippler et al. 2012; Raqib et al. 2009; Vahter 2009). The observed sex difference in tIgG in relation to arsenic exposure is in accordance with our previous studies, indicating that the association of prenatal arsenic exposure with acute respiratory infections was stronger in male infants than in female (Raqib et al. 2009). We also found that the adverse effects of maternal arsenic exposure during pregnancy on fetal growth and the genome-wide DNA methylation in cord blood were more pronounced in boys than in girls (Broberg et al. 2014; Kippler et al. 2012). The observed stronger association of arsenic

exposure with tIgG and tIgE in underweight children than in the children with adequate weight may be a consequence of the immunocompromised status of the malnourished children. It has been shown that malnutrition impairs host immune responses by decreasing cell-mediated immunity and increasing total immunoglobulins (Ozkan et al. 1993; Rodríguez et al. 2011)

The present study indicated an arsenic-induced reduction of mumps-specific IgG concentrations but this could be detected only in children with the lowest preimmunization plasma IgG. The suppressive effect seemed to start in early childhood and continued up to 9 years of age, one may speculate that this is due to arsenic-induced depletion of the antigen-specific T cells, resulting in reduced antigen-specific humoral immunity. However, we did not observe any indication of decreasing measles or rubella vaccine–specific IgG in relation to arsenic exposure, which was complicated by high preexisting specific IgG titers. In a previous study in Bangladesh, children with high total U-As (mean 291 μg/L, $n = 40$) had higher concentrations of IgG against diphtheria and tetanus, but not of measles-specific IgG, compared to children with very low U-As (mean 7 μg/L, $n = 20$) (Saha et al. 2013). It is, thus, possible that arsenic exposure differentially affects bacterial (e.g., diphtheria, tetanus, etc.) and viral (e.g., MMR) antigen-specific antibodies. Further in-depth studies are required to better understand the potential effects of arsenic on vaccine-specific immunity.

The strengths of our study include the population-based prospective design, relatively large sample size, availability of data on important covariates to adjust for possible confounding, and individual arsenic exposure assessment based on U-As concentrations once in early pregnancy (GW8) and twice during childhood (4.5 and 9 years of age). However, we could not perform a linear mixed model to evaluate the time-varying overall summary estimate given that we have outcome data (tIgG, tIGE, and tIgA) only in one time point, that is, at 9 years of age. Another strength of our study was the measurement of MMR-specific IgG before and after immunization, which allowed detection of a difference in mounting adequate response to a vaccine in relation to arsenic exposure. We used U-As for exposure assessment that reflects the exposure to inorganic arsenic from all sources, including food and water. One of the limitations of the present study was that MMR-specific IgG responses were measured after 21 days of immunization based on the fact that the half-life of plasma IgG is 21 days. However, all children might not have attained peak vaccine-specific IgG response within 3 weeks, as is generally expected within 4–6 weeks after immunization. Studies on vaccine response to measles have shown that at 21 days (3 weeks), 81% of vaccinees exhibit peak IgG responses (Helfand et al. 1999). Thus, it is likely that 21-days' vaccine response will be adequate to evaluate association between arsenic exposure and postimmunization response. Another limitation of the study was that we did not have reliable information on recent morbidity (past 1–2 months). However, we adjusted the regression models for plasma CRP concentrations as a marker of recent infections. Mumps and rubella were new vaccines for the study children because these vaccines were not included in the EPI at that time. However a large majority of the children had high preexisting antibodies, rendering the analyses of arsenic-related effects on vaccine-specific IgG titers difficult.

In conclusion, arsenic exposure appeared to modulate plasma tIgG and tIgE and to impair mumps vaccine–specific IgG response in the primary school-going children in rural Bangladesh. Such changes of humoral immune function have the potential to increase the susceptibility to infections, autoimmune diseases, and other chronic diseases in childhood and also later in life.

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